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RESEARCH ARTICLE

# First record of the moorland clouded yellow *Colias palaeno* (Linnaeus, 1761) (Lepidoptera: Pieridae) in Altai Krai (Russia, West Siberia) with notes on its DNA barcode

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## Abstract

*Colias palaeno* (Linnaeus, 1761) is reported from Altai Krai for the first time. The DNA barcode of the collected specimen was analysed and compared with molecular data on European populations of *C. palaeno* available in the public databases GenBank (http://www.ncbi.nlm.nih.gov) and BOLD (http://www.boldsystems.org). The molecular analysis has shown that the specimen from Altai Krai shares mitochondrial barcode with some specimens from mountain populations of the Alps and the Czech Republic, and differs significantly from lowland populations of *C. palaeno* from Central and Northern Europe.

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**Keywords**
New record, *Colias palaeno*, DNA barcoding, mitochondrial lineages, molecular analysis

## Introduction

The moorland clouded yellow, *Colias palaeno* (Linnaeus, 1761), is a Holarctic species distributed from Western and Central Europe, northern Fennoscandia, and Eastern Europe across Siberia to Mongolia, northeastern China, North Korea, Kamchatka, Sakhalin, Japan, and part of North America (Grieshuber et al. 2012). The species is highly endangered in Central Europe, where its range and abundance have shrunk dramatically during the XIX-XX centuries, and more widespread in Fennoscandia, European part of Russia and further east (Kramp et al. 2016). In the northern parts of its range, *C. palaeno* is distributed in lowlands, inhabiting mainly peat bogs and forest clearances associated with oligotrophic wetlands. At the southern edge of distribution, the species is predominantly montane-subalpine, and can be found in dwarf shrub heaths (Huemer 2004).

*C. palaeno* is characterised by strong intraspecific variability: specimens may significantly vary in size, differ in presence of discal mark on the forewings, width of black margin, upperside and underside wing colouration. Based on differences in external morphological characters and geographical distribution, numerous subspecies were described (Grieshuber et al. 2012). Several taxa (e.g. *C. p. aias* Fruhstorfer, 1903 from Japan, or *C. p. chippewa* Edwards, 1872 from North America) are considered as separate species by some authors (Grieshuber 2014, Maey 1986, Tuzov 1995, Verhulst 2000). Since the morphological traits differ significantly even within the same population, and many described subspecies of *C. palaeno* are known to intergrade, demonstrating intermediate phenotypes and clinal variation, their systematics and real taxonomic status (ecological forms, subspecies or species) are poorly understood and remain questionable.

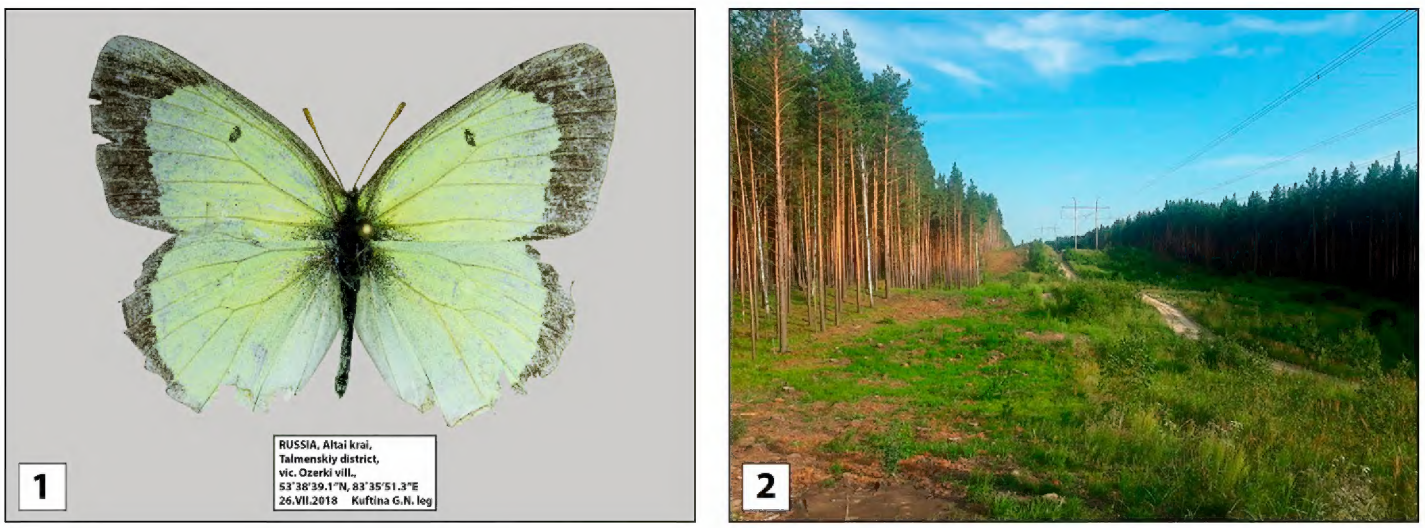
The fauna of Lepidoptera of South Siberia and the Altai-Sayan mountains has been intensively studied over the recent years (Ivonin and Nikolaev 2008a, 2008b, Tshikolovets et al. 2009, Yakovlev 2014). It is considered that two subspecies of *C. palaeno* are distributed in the region: the nominotypical and *C. p. orientalis* Staudinger, 1892; the western limit of the range of the latter lies approximately along the Yenisei River and the Altai Mountains (Grieshuber et al. 2012). Despite the presence of the species in question in adjacent territories (Novosibirsk and Kemerovo districts, Republic of Altai) and intensive studies of these regions, *C. palaeno* has never been reported from Altai Krai.

At the present time, molecular data (DNA barcodes) are available only for populations from Europe, Japan, and North America. The only comprehensive molecular study of the species in question made by Kramp et al. (2016) revealed complicated genetic structure of European *C. palaeno*. It was shown that specimens of *C. palaeno* from Europe represent nine haplotypes, clustering in four distinct clades

with genetic distances ranging from 1.17% to 9.17% (Kramp et al. 2016). In contrast with the populations from Europe, the genetic structure of the populations from European part of Russia, Siberia and Russian Far East still remains unknown. Here we report the first record of *C. palaeno* from Altai Krai and provide DNA barcode of the collected specimen.

## Material and methods

One worn male (GenBank accession number MT210322) was collected in the vicinity of Ozerki village, Talmenskiy district, Altai Krai, Russia [53°38'39"N 83°35'51"E], in a pine forest clearing on 26.07.2018 (Figs. 1–2).



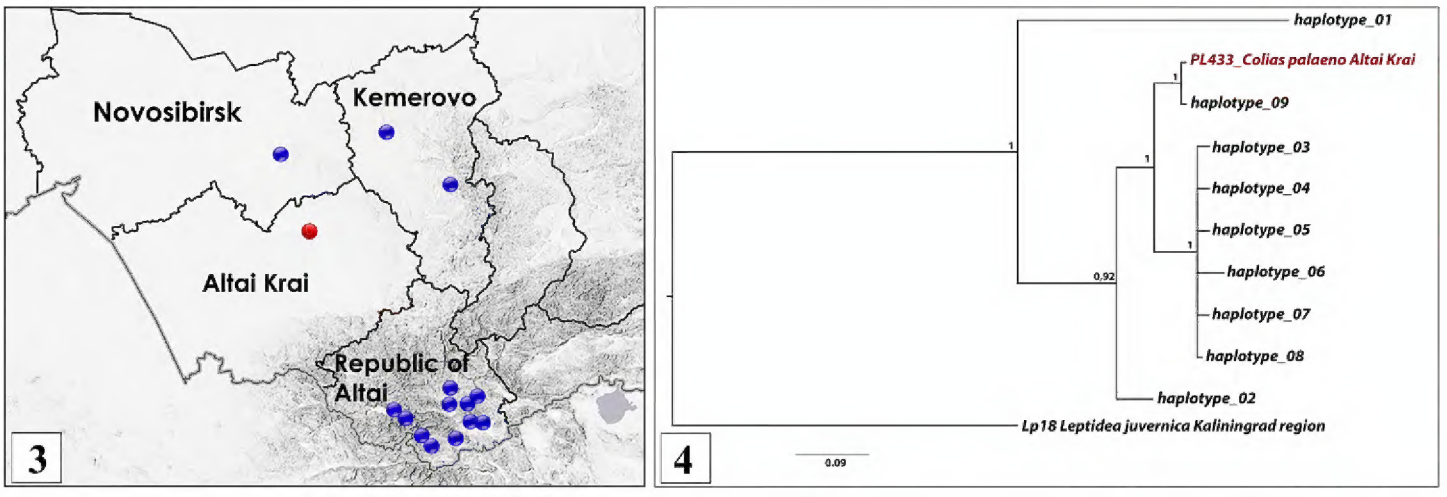

**Figures 1-2. 1.** *Colias palaeno*, male, vicinity of Ozerki village, Talmenskiy district, Altai Krai, Russia, [53°38'39"N 83°35'51"E], 26.07.2018. **2.** Collection site of *C. palaeno* in Altai Krai.

DNA extraction, amplification and further preparations for sequencing were conducted at "Taxon" Research Resource Center (http://www.ckp-rf.ru/ckp/3038/) of the Zoological Institute of the Russian Academy of Sciences (St. Petersburg). Sequencing of the standard DNA barcode (658 bp fragment of the mitochondrial cytochrome oxidase subunit I gene, COI) was carried out at the Research Resource Center for Molecular and Cell Technologies (St. Petersburg State University). We also include in analysis nine haplotypes obtained for Europe by Kramp et al. (2016) and available in GenBank (accession numbers KY204235, KY204236, KY204237, KY204238, KY204239, KY204240, KY204241, KY204242, KY204243). We used a sequence of the pierid *Leptidea juvernica* (Linnaeus, 1758) from Kaliningrad region (GenBank accession number MT210323) as an outgroup to root the phylogram. The data set of COI barcodes was analysed as described previously (Shapoval et al. 2017, Yakovlev et al. 2018). A Bayesian approach was used for estimating the phylogeny. Bayesian analyses were performed using the program MrBayes 3.1.2 with the nucleotide substitution model GTR+G+I. jModelTest was used to determine

optimal substitution models for Bayesian inference (BI) analysis (Posada 2008). TRACER, version 1.4 was used for summarizing the results of the Bayesian phylogenetic analyses (http://beast.bio.ed.ac.uk/Tracer).

## Discussion

The specimen of *C. palaeno* collected near Ozerki village is the first record of the species for Altai Krai. Other related records of *C. palaeno* attributed to the adjacent territories (Fig. 3): Novosibirsk district (vicinity of Berdsk – ca.110 km north of collection site in Altai Krai), Kemerovo district, and south part of the Republic of Altai (Tshikolovets et al. 2009). The revealed identity of the DNA barcode of the studied specimen with haplotype 9 (GenBank accession number KY204243), which comprises butterflies from the Alps (Austria) and the southwestern Czech Republic (Fig. 4). At the same time, the specimen from Altai Krai differs significantly from the haplotype 1, which consisted of specimens from Finland, Estonia, Latvia, Czech Republic, and Austria; from haplotype 2, which comprises butterflies from Baltic populations (Latvia and Estonia), and a cluster of six closely related haplotypes (3, 4, 5, 6, 7, 8), which unites butterflies from all over the European geographic range. The mean genetic distances were 7.33%±1.01%, 1.83%±0.52%, and 1.53%±0.46%, respectively.




**Figures 3-4.** **3.** Distribution of *C. palaeno* in Altai Krai (red circle) and adjacent territories (blue circles). **4.** Bayesian tree of *Colias palaeno* inferred from COI sequences. Numbers at nodes indicate Bayesian posterior probability. Scale bar = 0.09 substitutions per position.

Thus, the DNA barcode of *C. palaeno* from Altai Krai is appeared to be more closely related to barcodes of high-altitude specimens from the Alps rather than to those of lowland populations of Central and Northern Europe. Such an identity of geographically distant haplotypes may reflect ancient connections of populations of *C. palaeno* across Eurasia and their dispersal during the last glacial maximum. This phenomenon needs further exploration based on a broad phylogeographic analysis of haplotypes.

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## Authors contribution

G.N. Kuftina & N.A. Shapoval contributed equally to the study design, analysis and manuscript preparation.

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